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(54) Title: METHODS AND COMPOSITIONS FOR TARGETING SPECIFIC TISSUE

(57) Abstract

Methods and compositions for preferentially targeting the delivery of a substance to a sub-population of mammalian cells. The vehicle of the invention includes a targeting moiety capable of associating with or forming an envelope defining a compartment that contains the substance to be delivered to the sub-population of cells. The targeting moiety also contains a domain capable of interacting with a member of a binding pair located on the surface of the sub-population of cells.

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METHODS AND COMPOSITIONS FOR TARGETING SPECIFIC TISSUE

This invention was made with Government support under Grant No. AM-16666, awarded by the National Institute of Health. The Government has certain rights in this invention.

FIELD OF THE INVENTION

The invention is directed to methods and compositions for preferentially targeting the delivery of a substance, such as a nucleic acid, to specific tissue.

BACKGROUND OF THE INVENTION

In the field of gene therapy, viral vectors and liposomes have been proposed to introduce genes into cells (Williams, D.A. (1988) Hematology/Oncology Clinics of North America 2:277-287; and Mannino, R.J. et al. (1988) BioTechniques 6:682-690). Although such approaches may in some instances result in a localized administration of the vector or liposome, e.g., a viral vector administered via inhalation for localized delivery to lung tissue, a major drawback to such approaches is that there is no tissue-specific tropism. Thus, the DNA contained in the viral vector or liposome can be delivered non-specifically into many different types of cells.

Many genetic diseases are manifested in one tissue type and inserting genes non-specifically into many different cell types is not only unnecessary, but may be harmful under certain circumstances (Selden, R.F. et al. (1987) New Engl. J. Med. 317:1067-1076).

In addition, it is believed that most cancers originate from one cell type. At present, chemotherapy is the most widely-used approach to control cancer. Such chemotherapy, however, is not tissue specific in terms of delivery of the chemotherapeutic agent. Rather, the adverse effects

imparted upon the rapidly dividing cancer cells are also imposed upon normal cells.

Although one recent publication reported the use of a protein covalently coupled to DNA to target liver cells, this approach resulted in low efficiency in DNA uptake compared to the higher efficiency attainable with viral vectors (Wu, G.Y. et al. (1988) J. Biol. Chem 263:14621-14624).

Given the state of the art, it is apparent that a need exists for methods and compositions for targeting the delivery of various substances to specific cell types. Accordingly, it is an object of the invention herein to provide vehicles for targeting the delivery of a substance to specific cell types.

More particularly, it is an object of the invention to provide methods and compositions for targeting the delivery of a substance such as a nucleic acid or a therapeutic agent to a sub-population of mammalian cells contained within a living organism.

SUMMARY OF THE INVENTION

In accordance with the foregoing objects, the invention includes a vehicle for preferentially targeting the delivery of a substance to a sub-population of mammalian cells. The cell sub-population is characterized by the presence of a sub-population-specific first member of a binding pair on the surface of the cells. The vehicle comprises an envelope comprising a recombinant targeting moiety defining a compartment and a substance contained in the compartment. The recombinant targeting moiety comprises first and second domains. The first domain is capable of forming or associating with the envelope of the vehicle whereas the second domain is capable interacting with the first binding member on the surface of the sub-population of cells.

The invention also includes methods for preferentially targeting the delivery of a substance to the above sub-population of mammalian cells comprising contacting a

population of mammalian cells containing the sub-population with the above vehicle.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the construction of a hybrid erythropoietin-viral envelope gene.

FIG. 2 depicts the Western blot analysis of packaging cell sub-clones including the detection of the erythropoietin sequence.

FIG. 3 depicts the flow cytometric analysis of packaging cell lines based upon a detection of cell surface EPO epitopes by polyclonal anti-EPO antibodies.

FIG. 4 depicts the survivability of HeLa cells (wild-type or expressing the erythropoietin receptor) which have been exposed to neomycin or the retroviral vector encoding a neomycin resistant gene containing the EPO-*env* protein followed by a treatment with neomycin.

FIG. 5 depicts a similar experiment with NIH3T3 cells.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based upon a discovery that a virus containing a hybrid viral envelope protein containing an amino acid sequence corresponding to a portion of the sequence of erythropoietin is capable of preferentially infecting cells displaying the erythropoietin receptor on their surface.

As used herein, a "vehicle" of the invention is defined as any composition comprising an envelope defining a compartment and a substance contained therein that is capable of preferentially targeting the delivery of the substance to a specific sub-population of mammalian cells. The sub-population of mammalian cells is characterized by the presence of a first member of a binding pair on the surface of the sub-population. The vehicle is further defined by the envelope which comprises a "recombinant targeting moiety" capable of forming or associating with the envelope and which imparts cell specificity to the vehicle. In this regard, the targeting moiety contains at

least two domains. The first domain is capable of forming or associating with the envelope of the vehicle whereas the second domain is capable of interacting with the first member of the binding pair on the surface of the sub-population of mammalian cells.

In one embodiment of the invention the vehicle comprises a "viral vehicle". In an alternate embodiment the vehicle comprises a "liposome vehicle". The viral vehicles of the invention are generally formed from naturally occurring viruses and in particular those viruses capable of infecting eukaryotic cells, such as mammalian cells. As is well known, viruses contain a genome comprising either RNA or DNA which encode a variety of genes necessary for viral transfection and reproduction. Included within the viral genome are nucleic acid sequences encoding various enzymes, e.g., DNA or RNA polymerases and the like and structural proteins which surround the viral genome referred to as "viral coat proteins".

Some viral coat proteins make up that part of the virus particle known as the capsid. In addition, some viruses further contain a lipid bilayer surrounding the capsid which often contains viral envelope proteins. Depending upon the virus used and its envelope structure, i.e., capsid proteins only or capsid proteins in combination with membrane and envelope protein, the capsid protein or envelope protein is used to form the targeting moiety for the viral vehicle of the invention.

Since the targeting moiety contains a domain which is capable of forming or associating with the envelope of the virus vehicle, all or part of the viral coat protein is used to construct the recombinant target moiety. When only a portion of the coat protein is used, that portion of the coat protein which is necessary and sufficient for envelope formation is used. For viral envelope proteins, such necessary and essential features include the anchoring region and transmembrane region utilized by the envelope protein to display the envelope protein on an infected cell. When a virus containing an envelope protein is used,

it is preferred that the targeting moiety contain such anchor and transmembrane regions.

The second domain of the target moiety is capable of interacting with the first member of a binding pair which is on the surface of a sub-population of mammalian cells. As used herein, a "binding pair" includes receptor-ligand complexes, antigen-antibody complexes, enzyme-substrate complexes and the like. Thus, a sub-population of cells can be defined by the presence of one of the binding pairs on the surface of the cells of the population. For example, those cells containing an erythropoietin receptor define a cellular sub-population that can be targeted by utilizing erythropoietin (ligand) in the targeting moiety of the invention as the second domain capable of interacting with the erythropoietin receptor. Alternatively, the receptor can be used in the targeting moiety and the ligand displayed on the surface of the cells of the sub-population.

Similarly, a sub-population of cells can be defined by the presence of an antigen or antibody on the surface of the cells contained within that population. For example, a surface marker contained on the surface of a sub-population of cells can be used to generate monoclonal antibodies by methods well known to those skilled in the art. Monoclonal antibodies (especially those containing anchor and transmembrane regions or engineered through cloning and modification of cDNA to contain such sequences) can be used either as the targeting moiety alone (e.g., in a liposome vehicle) or in combination with a protein capable of associating with a viral surface. In an example of the latter case, the targeting moiety essentially comprises a chimeric antibody containing an anchoring and transmembrane region from a viral envelope protein coupled to at least the variable region of a heavy chain Ig molecule which is also associated with the light chain from the antibody. Alternatively, the vehicle of the invention can target a sub-population of lymphoid cells displaying a membrane bound form of an immunoglobulin. In such cases,

the antigen to the membrane bound antibody is used to form the second domain of the targeting moiety.

In general, the recombinant targeting moiety used to form the vehicle of the invention contains all or part of the second member of the binding pair. In some instances, the interaction between binding pairs is based upon an interaction involving a continuous epitope. In such cases, the minimal component of the second member of the binding pair that can be used in forming a targeting moiety consists of that epitope. In many instances, however, the interaction involves a discontinuous epitope. For example, in the case of proteins, two or more regions within the primary amino acid sequence of the protein are brought into close physical proximity in the tertiary structure of the protein to form the binding epitope. When only a portion of such a protein is used to form the targeting moiety, that portion of the primary amino acid sequence which encompasses such binding regions are preferably included in the targeting moiety so as to provide the strongest possible interaction with the other member of the binding pair located on the sub-population of mammalian cells.

The vehicle of the invention preferentially targets the delivery of substance to a sub-population of mammalian cells. Such preferential targeting is defined by comparing delivery of the substance to the sub-population of mammalian cells containing the first member of a binding pair as compared to a different population of a mammalian cell of the same species which does not display the first member of the binding pair. The increase in delivery as measured by binding of the vehicle to the sub-population or transferral of the substance of the vehicle into the sub-population cells, is generally greater than two-fold. However, as indicated in the examples, an increase in efficiency in infection by the viral vehicle disclosed therein ranged from 10-fold to about 30- to 40-fold as compared to wild-type cells not containing the receptor used in that experiment.

In an example herein, the recombinant targeting moiety comprises a carboxy terminal portion of the envelope protein of Moloney murine leukemia virus (MoMLV). The second domain comprises an amino terminal portion of erythropoietin. When the plasmid encoding this construct is expressed in a packaging cell line also capable of expressing the full length MoMLV envelope protein under conditions which provide for the formation of virus particles, a viral vehicle of the invention is formed. In this particular case, the recombinant targeting moiety is associated with the envelope (corresponding to the membrane and MoMLV envelope protein) defining a compartment that contains the remainder of the virus particle. As thus formed, the substance contained within the viral compartment can comprise protein surrounding the viral genome or the genome itself. In the former case, preformed proteins capable of being packaged with viral capsid proteins can comprise the substance contained in the compartment. In most embodiments, however, the substance of interest contained within such a viral vehicle is a recombinant DNA sequence introduced into the genome of the virus. Viral vehicles are preferred when the substance to be delivered to a sub-population of cells is a nucleic acid.

Thus, when used to treat a genetic disease, the skilled artisan identifies the defective gene and the cell type that expresses the normal wild type gene. Then, a surface marker is identified on the cell type normally expressing the wild type gene. The viral vehicle of the invention is then designed to contain a recombinant targeting moiety capable of forming or associating with the envelope of a selected virus such that the thus modified virus is capable of recognizing and binding the selected surface marker. In conjunction with the selection of the virus to be used in practicing the invention, the size of the recombinant nucleic acid to be incorporated into the viral genome is determined to facilitate the choice of virus to be used. The genome of the virus must be such that it can either

accommodate the selected recombinant nucleic acid or be modified to delete nonessential sequences such that the recombinant nucleic acid can be incorporated into the viral genome and still be packaged to form an intact viral particle containing the recombinant targeting moiety. In this regard, it is preferred that the genome of the wild type virus be modified not only to accommodate the recombinant nucleic acid to be incorporated but further to attenuate the virulence of the modified virus. Such modifications include but are not limited to the deletion of the viral genes encoding one or more DNA or RNA polymerases. Examples of viruses which can be used in practicing the invention include retroviruses, adenoviruses and adeno-associated viruses (Berkner, K.L. (1988) BioTechniques 6:616-629).

The Examples describe viral vehicles that demonstrate preferential targeting to cultured mammalian cells expressing the erythropoietin receptor. Thus, when used in vivo, the viral vehicle targets those cells within the organism expressing the erythropoietin receptor. A specific utility for such a vehicle is the treatment of hemoglobinopathies such as sickle cell anemia and β-thalassemia. These diseases involve a genetic defect wherein either an abnormal globin chain is made or little if any β-globin chain is produced. If a genomic clone encoding the β-globin gene is inserted into the genome of the virus vehicle in the Examples, the resulting virus vehicle is capable of preferentially recognizing not only red blood cells but also those precursors of red blood cells derived from hematopoietic stem cells that have committed to erythroid differentiation as evidenced by the display of the erythropoietin receptor. When the virus transfects such erythroid progenitor cells, the β-globin gene is integrated into the genome of the progenitor cells. As a consequence, a normal β-globin gene becomes integrated into the genome and is capable of modulating the disease by producing either normal amounts of β-globin or the wild type β-globin protein.

The viral vehicles of the invention can also be used to target the delivery of therapeutic agents to diseased cells such as cancer cells. For example, to the extent a surface marker is preferentially expressed on a cancer cell (i.e., solely on the surface of the cancer cell or at a level higher than that found on non-cancerous cell-types), an appropriate targeting moiety can be used to target a viral vehicle to bind to such preferentially expressed markers. The genome of the viral vehicle is recombined with a nucleic acid expression unit capable of expressing for example toxic polypeptides such as ricin, diphtheria toxin and the like. Upon recognition, binding and transfection into the cancer cell, the expression unit produces the toxic polypeptide to preferentially kill the cancer cell. In this regard, the expression unit comprises nucleic acid encoding the toxic polypeptide and one or more expression regulation sequences that are operably linked to the nucleic acid such that the nucleic acid is expressed in the transfected cancer cell so as to produce the toxic polypeptide. Such expression regulation sequences are preferably those which demonstrate tissue specific specificity to the cell type from which the cancer cell differentiated, more preferably an expression regulation sequence expressed exclusively by the cancer cell and most preferably the expression regulation sequence controlling the expression of the preferentially expressed surface marker.

In such embodiments, the preferred virus of choice is selected from the retrovirus family. Retroviruses are preferred because they require an actively dividing cell to efficiently integrate into the genome of its host. If non-cancerous cells also express the surface marker preferentially displayed on a cancer cell, the viral vehicle of the invention is also capable of recognizing, binding and transfecting such cells. This background level depends upon the amount of the surface marker found on such normal cells and the environment within which the marker is displayed. Further, to the extent that the viral vehicle

contains a normal viral coat protein, e.g., envelope protein, present in combination with the recombinant targeting moiety, the viral vehicle may also have the ability to non-specifically transfect cells independent of the presence of the surface marker utilized for recognition by the targeting moiety. When a retrovirus is used to practice the invention to treat cancer cells, however, such background infections in non-cancer cells are less likely to cause damage to normal cells that are not rapidly dividing since viral integration and expression of the toxic gene is less likely to occur.

The overexpression of oncogenes is often associated with the onset and development of cancer. In many instances, the oncogenes expressed encode receptors which are located on the surface of the cancer cell. For example, it has been recently disclosed that the proto-oncogene HER2 encodes a transmembrane tyrosine kinase whose overexpression has been correlated with several human malignancies, including breast, ovarian, gastric and endometrial cancers as well as non-small cell lung adeno carcinoma. See Holmes, et al. (1992) Science 256. As further disclosed, the protein heregulin-α has a single affinity binding site for tumor cell lines expressing the HER2 oncogene. The heregulin-α protein is therefore a candidate for use in treating the above identified cancers according to the teachings disclosed herein.

In addition to cancer cells, the invention can be used for treating autoimmune disease. For example, T-cell mediated autoimmune disease is characterized by a sub-population of T-cells containing a T-cell antigen receptor (TCAR) which is capable of recognizing and interacting with a self-antigen to elicit the autoimmune response. Experimental autoimmune encephalomyelitis (EAE) is a myelin basic protein induced demyelinating disease in mice. Recently, it has been shown that this disease is based, in part, upon the presence of a sub-population of T-cells containing well-defined variable regions containing specific segment subsets from the T-cell antigen receptor

repertoire. It has also been recently reported that mice treated with monoclonal antibodies specific to epitopes contained within the β-chain of the TCAR of the sub-population of T-cells responsible for the autoimmune disease is capable of preventing onset of the disease when the animal is challenged with myelin basic protein.

Since other T-cell-mediated autoimmune diseases are believed to form the basis of various autoimmune diseases, especially in humans, the vehicle of the invention can be used as an alternate approach to treating or preventing such autoimmune diseases. In such cases, a monoclonal antibody specific for the TCAR responsible for the autoimmune disease is used to form the vehicle of the invention. When a viral vehicle is used, the substance contained within the virus is preferably a toxic gene capable of being expressed when transfected into those T-cells containing the autoimmune inducing TCAR. Alternatively, when a liposome is used, the substance contained therein can be a nucleic acid containing an expressible gene encoding a toxic polypeptide or a therapeutic agent such as a chemotherapeutic agent capable of killing the cell, e.g., the ricin or diphtheria toxin proteins.

In addition to viral vehicles, liposome vehicles can be used to practice the invention. Liposomes are well known to those skilled in the art and generally comprise membranous vesicles containing lipid bilayers which are capable of encapsulating various drugs or other chemicals. See, e.g., U.S. Pat. Nos. 4,053,585; 4,397,846; 4,411,894; 4,427,649, and Papahadjopolous, et al. (1967) Biochem. Biophys. Acta. 135:639; Bangham, J. et al. (1965) J. Mol. Biol. 12:238, 252; Bapzri and Korn (1973) Biochem. Biophys. Acta. 298:1015; and Miyamato, et al. (1971) "Preparation and Characteristics of Lipid Vesicles", J. Membrane Biol. 4:252-269.

When a drug or other chemical is incorporated into a liposome, that substance is generally included in the reaction mixture used to synthesize the liposome.

Accordingly, in practicing the invention to form liposome vehicles, the substance to be contained in the compartment of the liposome vehicle, e.g., nucleic acid, drug or therapeutic agent, is included during liposome synthesis. Further, the targeting moiety of the invention is also included during the formation of the liposome.

When using a liposome, the targeting moiety preferably contains a anchoring region and a transmembrane region, e.g., from immunoglobulin or other membrane bound protein, so as to associate with the lipid bilayer of the liposome. It, of course, also contains the domain capable of interacting with the first member of the binding pair contained on the sub-population of mammalian cells. A preferred targeting moiety comprises a monoclonal antibody (preferably IgM) specific for a cell surface antigen or an antibody that has been engineered through cloning and modification to contain an anchoring and transmembrane region.

Since the targeting moiety is not produced within the liposome but rather is incorporated into the liposome membrane during liposome formation, the thus formed liposome contains targeting moiety wherein the domain capable of interacting with the first binding member is displayed on the inner and outer surface of the liposome membrane.

The following is presented by way of examples and is not to be construed as limitation of the scope of the claims appended hereto.

Example 1

Construction of hybrid EPO-*env* genes

This example describes the construction of hybrid erythropoietin + viral envelope (EPO-*env*) genes in eukaryotic expression vectors.

The entire wild type moloney murine leukemia virus (MoMLV) envelope gene (*env* gene; from position 5760 to position 7820 of the retroviral genome; Shinnick, T.M. et al. (1981) Nature 293:543-548) was cloned into a pBR322-

based plasmid vector. Transcription of the *env* gene is driven by the 5'-MoMLV-long terminal repeat sequence (LTR). The *psi* (packaging signal) sequence was deleted, but the vector retained the 5' and 3' splicing signals for the *env* mRNA. The intervening *gag* and *pol* structural genes, which are normally spliced out of the *env* message (Weiss, R., N. Teich, H. Varmus, J. Coffin, eds. (1984) "RNA Tumor Viruses: Molecular Biology of Tumor Viruses", 2d edition, Cold Springs Harbor Laboratory, Cold Springs Harbor, NY), were deleted, thus leaving only a small sequence in between the splicing signals remaining to be spliced.

Portions of the envelope were then selected for removal and replaced with the EPO coding sequence. In the center of the outer protein subunit (gp70) of the envelope lies a proline-rich region which is a likely candidate domain for cell receptor recognition (Koch, W. et al. (1983) J. Virol. 45:1-9). Other sites include two regions which flank a region of homology in the amino-terminal portion of the molecule (Mark, G.E. et al. (1984) J. Virol. 49:530-539), and sites at which point mutations give rise to a paralytogenic mutant of MoMLV (Szurek, P.F. et al. (1988) J. Virol. 62:357-360). These regions are target sites for replacement with EPO. At the same time, portions of the gp70 sequence which encode the *env* signal peptide at the amino terminus, as well as the cysteine residues in the carboxyl-terminal region which participate in sulfhydryl bonding with the inner envelope subunit (p15), were left intact (Shinnick, T.M. et al. (1981) Nature 293:543-548). Unique restriction sites exist at position 5923 (BstEII) and position 6537 (BamHI). Deletion of the envelope sequence between these two sites preserves the *env* signal sequence and allows the EPO sequence (approximately 500 base pairs in length) to be inserted at the amino-terminal end of gp70.

A second EPO-*env* hybrid was also constructed to insert the EPO sequence in a more central location, directly overlapping the proline-rich region. Since no convenient restriction sites exist in this region, unique sites were

created at positions 6250 and 6750 by means of PCR-mediated mutagenesis. These new restriction sites were used to delete the intervening *env* sequence and insert the EPO sequence.

The EPO cDNA sequence coding for the mature 166-amino acid peptide hormone without the 27 amino acid signal peptide (Jacobs, K. et al. (1985) Nature 313:806-810; and Lin, F.K. et al. (1985) Proc. Natl. Acad. Sci. USA 82:7580-7584), was used to replace the MoMLV *env* sequences (Shinnick, T.M. et al. (1981) Nature 293:543-548) which had been deleted in the amino-terminal end and the central region of the envelope protein. PCR-mediated mutagenesis (Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., eds. (1990) "PCR Protocols: A Guide to Methods and Applications", Academic Press, San Diego, CA) was used to create compatible ends on the EPO sequence, so that it could be inserted via the appropriate restriction sites while maintaining the proper reading frame (Figure 1).

Example 2

Construction of packaging cell lines containing the EPO-*env* genes

The EPO-*env* constructs were transfected into the cell line PA317 (Mann, R. et al. (1983) Cell 33:153-159; and Cone, R.D. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6349-6353). The PA317 cell line is a derivative of NIH3T3 cells into which MoMLV genes have been stably integrated; the PA317 cells possess the *gag* and *pol* genes as well as the amphotropic *env* gene, but the *psi* packaging signal sequence is deleted. Thus, these cells produce viral proteins without assembling them into wild type (endogenous) virus, and can be used to package exogenous viral vectors which do contain the *psi* sequence into virions with an amphotropic (i.e., capable of infecting both murine and non-murine cells).

The EPO-*env* plasmids were co-transfected into the PA317 cell line, along with a methotrexate-resistant dihydrofolate reductase (DHFR) gene as a selectable marker

(Simonsen, C.S. et al. (1988) Nucleic Acids Res. 16:2235-2246), using the calcium phosphate precipitation method (Wigler, M. et al. (1979) Cell 16:77). After transfection, the cells were selected for stable integration of the *env* sequences in medium containing methotrexate. Cells which had stably integrated the sequence into their genomes were methotrexate-resistant and therefore survived in this medium, growing as isolated colonies when the plating density was low enough. Stable clones were picked in this manner and grown in separate wells.

After stably transfected sub-clones were isolated, Western blot and flow cytometric analyses were performed to confirm that the recombinant envelope proteins were being expressed and that they were reaching the surface of the packaging cell. Monoclonal anti-EPO antibodies and biotinylated secondary antibodies were used to detect expression in Western blots using a chemiluminescence-based technique (Linscott, W.D. (1990) Linscott's Directory of Immunological and Biological Reagents, 6th ed., Mill Valley, CA), and the EPO-*env* hybrid protein was detected as an intact 70 kiloDalton band in some sub-clones, but seemed to have been deleted or rearranged in others (Figure 2). Using the parental cell line PA317 as a negative control, polyclonal anti-EPO-antiserum and fluorescein-labeled secondary antibodies were used to detect EPO expression on the cell surface of hybrid EPO-*env*-containing cell lines by flow cytometry (Figure 3).

Example 3

Packaging of retroviral vectors

This Example discloses the use of the cell lines of Example 2 to package replication-defective retroviral vectors containing the neomycin-resistance (neo^R) gene and the β-galactosidase (β-gal) gene.

Stable transfectants producing high levels of recombinant envelope proteins were transfected, by means of the calcium phosphate precipitation method, with replication-defective retroviral vectors which contain the

psi-packaging signal, but whose *gag*, *pol*, and *env* genes have been deleted and replaced with genes encoding neomycin resistance (neo^R) and β-galactosidase (β-gal). These vectors were also transfected into the parental wild type amphotropic packaging cell line PA317 as a control. The cell culture supernatant containing these transiently expressed viruses was harvested, filtered to remove cellular debris, and used to infect target cells with or without the EPO receptor.

Example 4

Tissue specificity and infection efficiency of the packaged virus

The target cells consisted of four types: (1) wild type HeLa cells, which are non-murine and do not express the EPO receptor (D'Andrea, A. et al. (1989) Cell 57:277-285), (2) HeLa cells which have been stably transfected with the EPO receptor, (3) wild type NIH3T3 cells, which are murine fibroblasts and do not express the EPO receptor, and (4) NIH3T3 cells which have been stably transfected with the EPO receptor.

HeLa and NIH3T3 cells were co-transfected with the EPO receptor cDNA cloned into an appropriate eukaryotic expression vector containing a methotrexate-resistant DHFR gene (Simonsen, C.S. et al. (1988) Nucleic Acids Res. 16:2235-2246). After selection with methotrexate, the surviving colonies were isolated and expression of the EPO receptor was determined by a radioligand (^{125}I-EPO) binding assay (D'Andrea, A. et al. (1989) Cell 57:277-285). The sub-clone showing the highest levels of EPO receptor expression was selected and further grown in medium containing increasing concentrations of methotrexate, in order to further amplify the expression of EPO receptor. Receptor levels in these cells was calculated to be on the order of about 1000 receptors per cell. As an additional positive control prior to the infection experiments, the Neo^R/β-gal vector alone, (unpackaged) was transfected into both cell types by calcium phosphate precipitation, to

confirm that the neomycin-resistance gene, whose expression is directed by the MoMLV LTR, is expressed efficiently in these cells.

All cells exposed to virus were subjected to selection with neomycin, and the surviving colonies were examined and counted after Giemsa staining. There proved to be an increased efficiency of infection when viruses expressing the recombinant EPO-*env* protein were contacted with cells bearing the EPO receptor, as compared to cells without the receptor. This increased efficiency was about 10-fold in the case of HeLa cells with EPO receptor (Figure 4), and 30- to 40-fold in the case of NIH3T3 cells with EPO receptor (Figure 5), compared to their respective wild type cells without the receptor.

Having described the preferred embodiments, it will be apparent to those skilled in the art that various modifications can be made to the embodiments disclosed herein and that such modifications are intended to be within the scope of the invention.

WHAT IS CLAIMED IS:

1. A vehicle for preferentially targeting the delivery of a substance to a sub-population of mammalian cells characterized by the presence of a first member of a binding pair on the surface of said sub-population, said vehicle comprising an envelope comprising a recombinant targeting moiety defining a compartment and a substance contained in said compartment, wherein said targeting moiety comprises first and second domains not naturally associated with each other and wherein said first domain forms or associates with the envelope defining said compartment and said second domain comprises a second member of said binding pair or portion thereof which targets said vehicle to said sub-population of mammalian cells.

2. The vehicle of Claim 1 wherein said vehicle is capable of transferring said substance into said cell.

3. The vehicle of Claim 1 wherein said substance is a therapeutic agent.

4. the vehicle of Claim 3 wherein said therapeutic agent is a nucleic acid.

5. The vehicle of Claim 2 wherein said substance comprises a recombinant viral genome.

6. The vehicle of Claim 1 wherein said envelope comprises a membrane.

7. The vehicle of Claim 1 wherein said envelope comprises a viral coat protein.

8. The vehicle of Claim 7 wherein said viral coat protein comprises a viral envelope protein.

9. The vehicle of Claim 7 wherein said first domain of said targeting moiety comprises a portion of a viral coat protein.

10. The vehicle of Claim 9 wherein said viral coat protein comprises a viral envelope protein.

11. A method for preferentially targeting the delivery of a substance to a sub-population of mammalian cells characterized by the presence of a specific surface receptor for a ligand, said method comprising contacting a population of mammalian cells containing said sub-population with any one of the vehicles of Claims 1 through 10 and 12 wherein said receptor and said ligand comprise said first and said second members of said binding pair.

12. The vehicle of Claim 1 wherein said binding pair comprises erythropoietin and erythropoietin receptor.

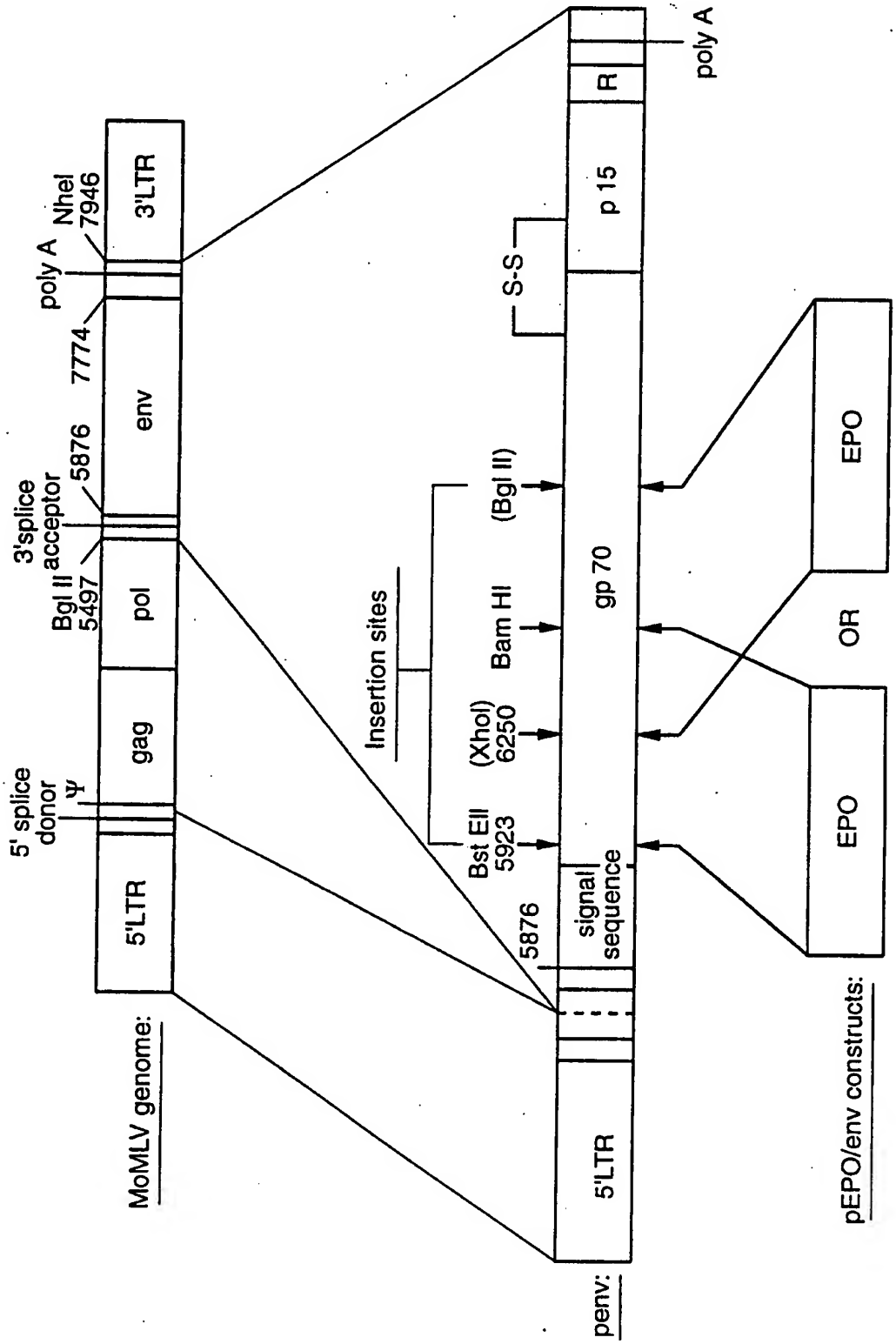
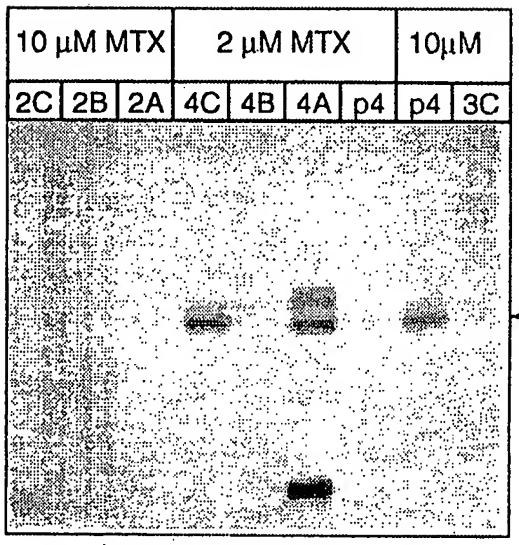

MoMLV genome:
5'LTR
gag
pol
env
3'LTR
5' splice donor
Ψ
3'splice acceptor
Bgl II 5497
5876
7774
poly A
NheI 7946
Insertion sites
Bst EII 5923
(XhoI) 6250
Bam HI
(Bgl II)
S-S
signal sequence
5876
p 15
R
poly A
gp 70
penv:
5'LTR
pEPO/env constructs:
EPO
OR
EPO

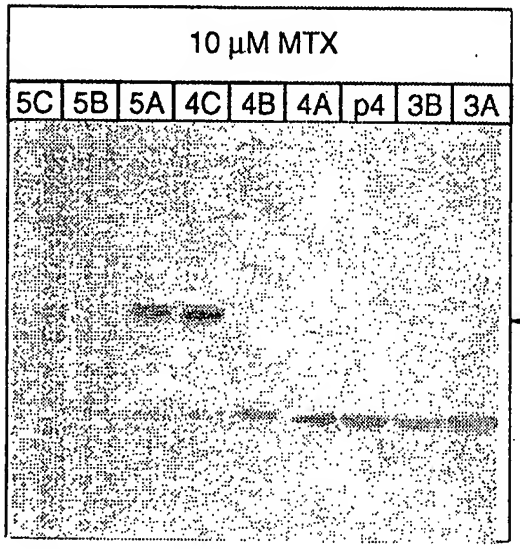
FIG._1

FIG._2A

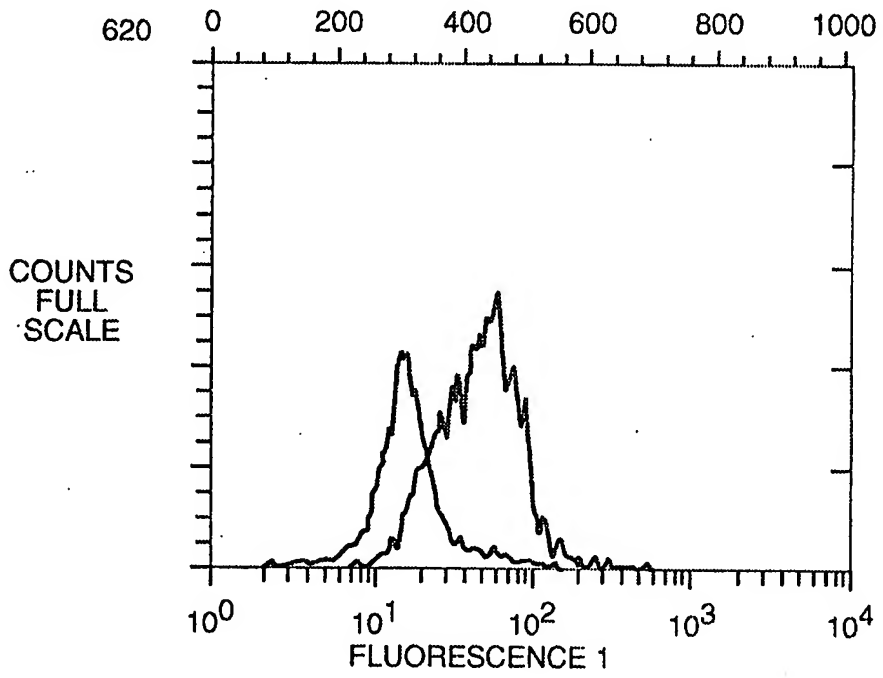


EPO / env (N - terminal 5923 position)

FIG._2B

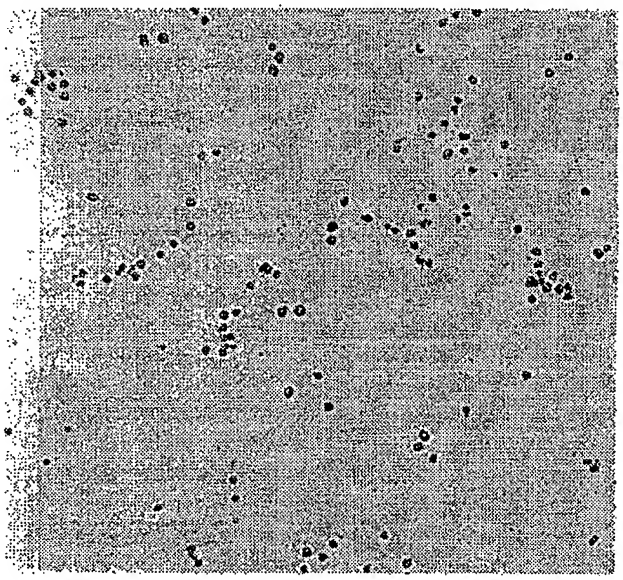


EPO / env (central 6250 position)



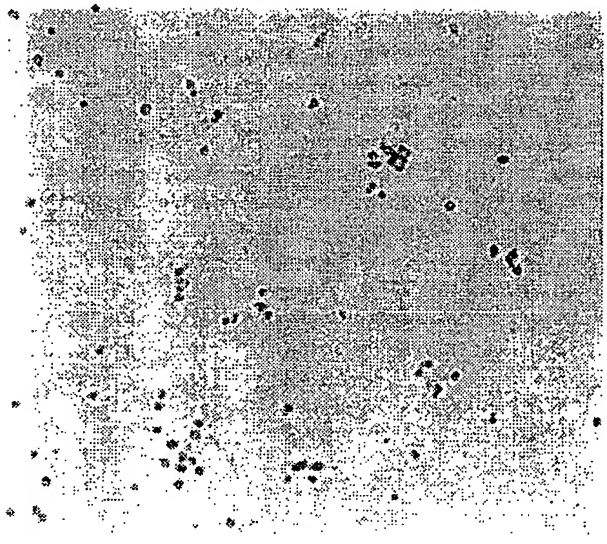
PA317 (wild type) vs. PA317 + EPO / env

FIG._3



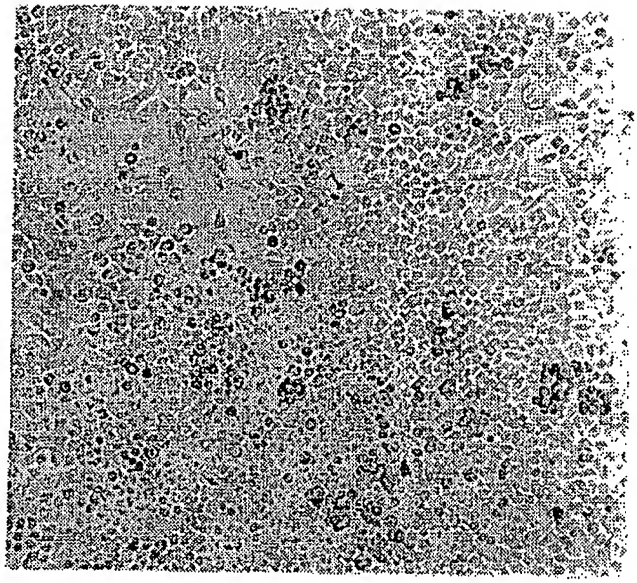
HeLa + EPO - R
(negative control)

FIG._4B



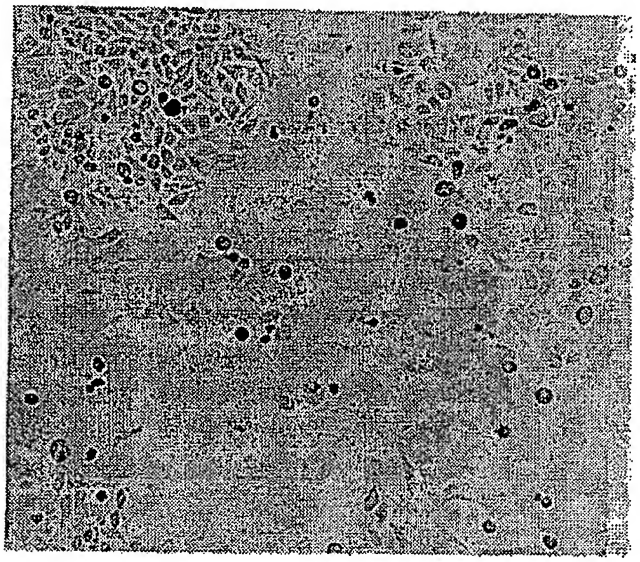
wt HeLa
(negative control)

FIG._4A



HeLa + EPO - R
(EPOenv virus)

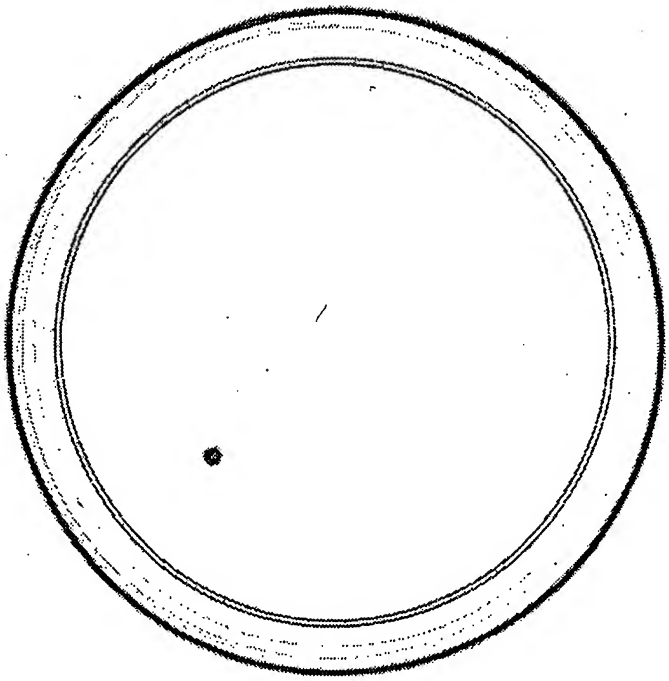
FIG._4D



wt HeLa
(EPOenv virus)

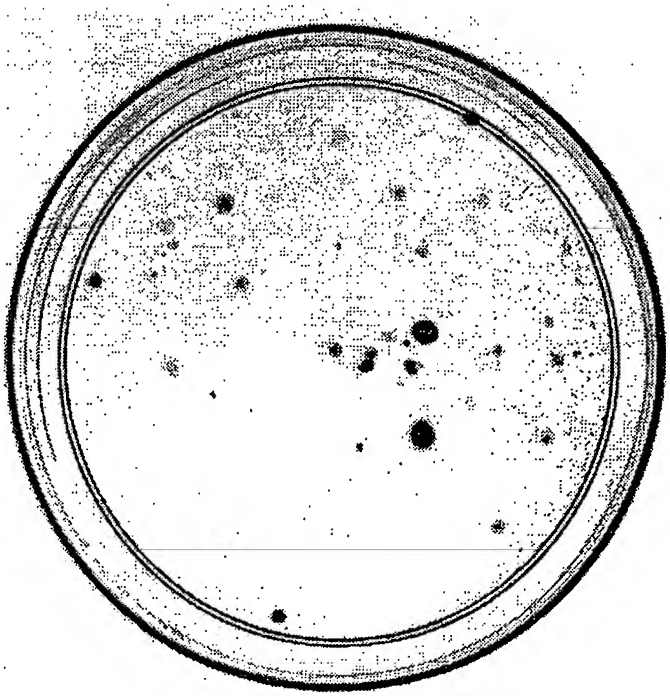
FIG._4C

FIG._5A



NIH3T3 wild type
(EPOenv virus)

FIG._5B



NIH3T3 + EPO receptor
(EPOenv virus)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/05260

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/12; A61K 39/00; CO7K 3/00; CO7K 13/00
US CL :424/88, 89; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, search terms:targeting, vehicle, composition, viral protein, erythropoietin, therapeutic, substance, receptor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Nature, Volume 339, issued 01 June 1989, Evans, et al, "An Engineered Poliovirus Chimaera Elicits Broadly Reactive HIV-1 Neutralizing Antibodies", pages 385-388, see entire article.	1-11 12
Y	Proceedings National Academy of Sciences, Volume 82, issued November 1985, Lin et al, "Cloning and Expression of the Human Erythropoietin Gene", pages 7580-7584, especially page 7581.	12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
22 July 1993

Date of mailing of the international search report
02 AUG 1993

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/05260

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Nature, Volume 313, issued 28 February 1985, Jacobs et al, "Isolation and Characterization of Genomic and cDNA Clones of Human Erythropoietin", pages 806-810, especially pages 806-807.	12